4. Fairley, G. B., Duggan, T. L., Stormont, R. T., and Pfeiffer, C. C.: The use of penicillin in the treatment of peritonitis, J. Am. Med. Assn., 126:1132 (Dec. 30), 1944.

5. Herrin, R. C., and Meek, W. J.: Distention as a factor in intestinal obstruction, Arch. Int. Med., 51:152 (Jan.), 1933.

6. Kay, John Howard, and Lockwood, John Salem: Experimental appendical peritonitis. I. The prognostic significance of certain hemotologic factors especially the prothrombin time, Surgery, 20:56 (July), 1946.

7. Kay, John Howard, and Lockwood, John Salem: Experimental appendical peritonitis. II. The significance of imbalance of circulating fibrinolytic and antifibrinolytic factors in the course of the disease, Surgery, 21:155 (Feb.), 1947.

8. Miller, Philip C.: Protection against bacterial endotoxins by penicillin and its impurities, Science, 107:118 (Jan. 30), 1948.

9. Murphy, John J., Ravdin, Robert G., and Zintel, H. A.: The use of streptomycin in experimental peritonitis, Surgery, 20:445 (Oct.), 1946.

10. Pulaski, E. J., and Sprinz, H.: Streptomycin in surgical infections; laboratory studies, Ann. Surg., 125:194 (Feb.), 1947.

11. Schneider-Green, J. E., and Houston, J. M.: The subcutaneous administration of penicillin, J. Roy. Army Med. Corps, 85:234 (Nov.), 1945.

12. Stansly, P. G., Sheperd, R. G., and White, H. J.: Polymyxin: A new chemotherapeutic agent, Bull. Johns Hopkins Hosp., 81:43 (July), 1947.

13. Tashiro, S., and Zinninger, M. M.: Appendicitis: A review of 936 cases at the Cincinnati General Hospital, Arch. Surg., 53:545 (Nov.), 1946.

14. Van Zwalenburg, C. V.: Strangulation resulting from distention of hollow viscera, Ann. Surg., 46:780 (Nov.), 1907.

15. Ward, R.: Apparatus for continuous gastric suction or duodenal lavage, J. Am. Med. Assn., 84:1114 (April 11), 1925.

The Role of Tissue Permeability With Particular Reference To the Blood-Brain Barrier in Diseases of the Central Nervous System

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THE attention of certain of my associates and myself has been directed both experimentally and clinically for several years to the possibility that permeability plays a role in disease processes of the central nervous system. We have been particularly interested in the blood-brain barrier and have developed methods for studying the permeability of this neurophysiological mechanism.

We have been able to show that the locus of this barrier resides, as Spatz[13] postulated, in the endothelial cells lining the walls of the cerebrovascular tree. Using the supravital dyes, Brilliant Vital Red and Trypan Red,[2] which uniquely stain these cells and which do not pass beyond the blood vessels to gain entrance into the nerve tissue proper, we have been able to lower the permeability of the blood-brain barrier and to prevent abnormal increases in the permeability of this barrier such as occur in many diseases of the central nervous system and which we have been able to produce in certain instances under controlled conditions in the experimental laboratory.

The neurophysiologic role of the blood-brain barrier is presumably fundamental in maintaining the homeostasis and conditions for normal metabolism within the central nervous system. The barrier also plays an important role in protecting the brain in various toxic conditions. For example, the barrier normally is impermeable to such electro-negative bacterial toxins as tetanus, diphtheria, botulinus and staphylococcus and such chemical agents as thiocyanates, neoarsphenamine, iodides and bromides.[10]

Studies on the blood-brain barrier indicate that practically all disease processes of the central nervous system tend to break the barrier down, that is, abnormally increase the permeability of the barrier. The results of the numerous studies[11] along this line may be listed in the following categories:

1. *Inflammatory Processes*
   - Meningitis
   - Encephalitis
   - Virus Infections
   - Abscess
   - Injury
2. *Toxic Processes*
   - Eclampsia
   - Acute Alcoholism
   - Theophyllin
   - Urotropin
   - Potassium Bromide
   - Urea
   - Bile Salts
   - Sodium Salicylate
3. *Physiologic Conditions*
   - High and low pH
   - High and low osmotic pressure
   - Menstruation
4. *Other Diseases of the Central Nervous System*
   - Tumors
   - Gliosis
5. *Tests and Surgical Procedures*
   - Craniotomy
   - Pneumoencephalogram
   - Lumbar puncture with forced drainage of cerebrospinal fluid

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Although the techniques and methods used in the majority of these studies may be criticized, the results in many studies and especially in those which have employed dyes, indicate that almost any disturbance within the central nervous system is associated with an increase in the permeability of the blood-brain barrier.

Our own studies have been directed along the following lines: to study the role of permeability in

1. Toxic diseases of the central nervous system.
2. Degenerative diseases of the central nervous system of possible toxic origin.
3. Cerebral concussion and other post-traumatic conditions.
4. Convulsive states.
5. Electroshock therapy.
6. Cerebro-vascular disorders.

Both experimental and clinical studies have been performed along these various lines. The traumatic and electroshock studies, for example, have been largely experimental, while the degenerative studies have been largely clinical. In all instances, however, the fundamental experimental observation that the permeability of the blood-brain barrier could be lowered by the supravital dyes, Brilliant Vital Red[1] and Trypan Red[2] has been used as a mechanism to alter the permeability in each condition studied and to test the relationship between permeability and other findings characteristic of the condition. For example, in using triphenyl phosphite as a convulsive agent in experimental epilepsy[3] we found that this compound was hydrolyzed readily in vivo and that two effects resulted from its breakdown products. The phenol fraction produced early convulsive effects at the cord level and the phosphorous acid fraction was selectively and preferentially absorbed by the gray matter of the central nervous system and caused late degenerative changes in the nervous system similar to such other esters of phosphorous acid as triorthocresyl phosphite, which produces a combined system degeneration of the spinal cord, and triorthocresyl phosphate, the contaminant responsible for the degenerative changes in cases of Jamaica ginger paralysis. If the animals were previously stained with Brilliant Vital Red, these toxic effects could be prevented. This illustrates our use of the dyes in toxic conditions of the central nervous system.

Our interest in the degenerative diseases of the central nervous system was stimulated by the concept that certain of these unexplained conditions might have a toxic origin. For example, this theory has been postulated for amyotrophic lateral sclerosis. Since the supravital dyes can be used with safety in man, we felt justified in trying them in these otherwise hopeless conditions with which we are so frequently confronted in neurologic practice. Such studies have been done without promises and only after a frank discussion of the situation with the patient and his family which would assure us of their cooperation in the prolonged follow-up that is essential in these conditions. Aside from testing the value of the dye therapy in these cases, we hoped that we might begin to sort out the large group of degenerative diseases and segregate those of toxic origin from the rest of the group.

Reference will be made only to one member of this group—amyotrophic lateral sclerosis.[4] Our results have been classified on a four-point scale:

1. No objective effect but possible subjective improvement.
2. Diminution of fasciculation.
3. Suggestive slowing of disease.
4. Process arrested and possible improvement in strength and weight.

On this basis, fasciculations were diminished in the majority, progression of the disease process was suggestively slowed in nearly 50 per cent and about one patient in six obtained an apparent arrest or even some improvement.

Although no final conclusions may be drawn from such data, the relationship between treatment and improvement in several patients appeared more than coincidental, especially in those patients in whom a recurrence of the disease process, following a discontinuance of the treatment, was again modified when adequate dye therapy was resumed. We have felt that the results were sufficiently encouraging to warrant further trial. Tentatively the same may be said for progressive muscular atrophy and progressive muscular dystrophy. Because of the variable and remissive course, characteristic of multiple sclerosis, it is impossible to arrive at any early conclusions in this condition. Our experience in other, more rare diseases of the central nervous system is too limited to permit any conclusions. Final conclusions as to the value of the dye therapy in these conditions and as to the significance of the results in establishing a toxic origin of them must await further trial and experience.

Our interest in cerebral concussion was stimulated by the concept that the maximal effect of a transmitted concussive blow might be expected at those points at which abrupt changes occur in the structure and density of the brain.[5,6] Shearing forces are known to produce their greatest effect at such junction points. Considering the anatomy of the brain, one would not expect major changes between cellular and extracellular elements, that is, along the cell membranes of the relatively homogeneous masses of the cerebral cortex. Where the cellular masses of the brain join other structures of different histological character, which are relatively large and fixed, such as the ependymal lining of the ventricular spaces and especially where the cellular masses of the brain make contact with their supporting vascular tree, one might expect greater shearing forces and neurophysiological changes. Disrupting effects at this latter point might be reflected in changes of vascular permeability, perivascular edema and nutritional alterations in the neighborhood effecting cerebral metabolism. Depending upon its severity and duration, neurophysiologic changes and even irreversible, degenerative changes might be produced. The perivas-

cular pathological changes described by Scheinker and Evans,[12,9] in more severe traumatic lesions of the brain would be in keeping with this hypothesis.

With this concept in mind, we have studied the effect of concussion on the vascular structure of the brain and have done so in experiments aimed to give us information on the permeability of the blood-brain barrier. The studies involved determinations of the distribution of cocaine by spectrophotometric methods in the brain and blood following the intravenous administration of this drug in control and traumatic groups of cats. As intimated, the object of these experiments was to determine whether or not alterations in the concentration of cocaine occurred in the central nervous system following cerebral concussion, which might reflect permeability effects of possible neurophysiologic and etiologic importance in the post-concussional state and post-traumatic head conditions.

Significant rises in the concentration of cocaine were found in the cerebral cortex after concussion without appreciable alterations in the blood concentration levels. These results indicate that cerebral concussion increases the permeability of the blood-brain barrier. Similar studies following preliminary injections of Trypan Red showed concentrations of cocaine in the post-concussional cortex which were equal to or lower than the corresponding control values, while again the blood concentration levels remained the same. It was concluded that Trypan Red effectively counteracted the permeability effect of cerebral concussion on the blood-brain barrier. It is also of interest in these studies that while approximately 80 per cent of the cats showed a cerebral dysrhythmia by electroencephalography three days after cerebral concussion, following preliminary Trypan Red injections the dysrhythmia either did not appear or appeared only in minor degree three days after traumatization. Since Trypan Red appears to counteract both the increase in permeability of the blood-brain barrier and the cerebral dysrhythmia associated with cerebral concussion, this dye may prove of practical value in determining the possible relationship between these neurophysiologic phenomena and the clinical symptoms characteristic of the post-concussional state. Inasmuch as Trypan Red may be used with safety in man, such therapeutic possibilities appeared worthy of exploration. Preliminary clinical studies, which are at present under way, suggest that this agent may have some beneficial effect on the post-concussive state.

Our interest in the use of the supravital dyes in the convulsive states was initiated by the discovery of Cobb and his co-workers[8] that Brilliant Vital Red would protect in both toxic epilepsy under controlled experimental conditions and also in human convulsive states. We have confirmed the observations of Cobb in both experimental and human epilepsy[1] and have continued to use this method of treatment as an adjunct to other anti-convulsive therapy when more routine methods have not proven adequate to control the convulsive state. Although not as potent as some of the modern anti-convulsive drugs now available, it is a valuable agent in those patients not fully protected by the usual methods, since it can be used in conjunction with them. We have also found that Trypan Red[2] is, in general, as effective as Brilliant Vital Red and because of its lower solubility and the fact that it is excreted more slowly than Brilliant Vital Red, it has proven of more practical value in actual practice.

The implications of the effectiveness of the supravital dyes in epilepsy are interesting and important. Why should an agent which alters the permeability of the blood-brain barrier and which does not itself gain entrance to the nerve tissue proper, have any effect on the convulsive state? The answer must be sought in the effect of the blood-brain barrier upon the physiological and biochemical state of the brain and consequently its effect in modifying the reactivity of the brain. When one considers the basic mechanisms known to modify the convulsive state, such as the acid-base balance and water balance of the central nervous system, it becomes apparent that many mechanisms which lower the permeability of tissue also afford protection in the convulsive states and, vice versa, that factors which increase permeability also increase convulsive susceptibility. Although the alteration of permeability in itself is presumably of the greatest importance in epilepsy induced by drugs, it is conceivable that some other changes associated with this alteration may be the precipitating factor in human epilepsy. Numerous complex physiologic and biochemical changes are associated with an alteration of the semipermeable characteristics of tissue membranes, and since these changes occur together, it is not possible to say that any single factor or combinations of factors is responsible for any resulting physiological effect. It seems likely that the convulsive state is a result of the whole complex of changes, that is, the changes associated with increased permeability in cortical tissue cause a more unstable and irritable state which, in turn, is characterized by an increased susceptibility to convulsions. As has been pointed out, those mechanisms, which are known to increase the permeability of tissue, such as alkalosis, hydration, anoxemia and inflammatory changes, are also known to lower the convulsive threshold. As a single mechanism of fundamental neurophysiologic importance in determining cellular nutrition and reactivity, permeability, or rather the complex of changes associated with alterations of permeability, offers an attractive hypothesis for the numerous, and otherwise unrelated, factors known to be of importance in modifying convulsive reactivity.

More recent investigations, possibly related to our earlier studies on the convulsive states, have been conducted in an attempt to elucidate the neurophysiologic basis of electroshock therapy.[7] Experimental studies with electroshock have been disappointing in that they have failed to explain the basis for the beneficial effects of this form of therapy in psychiatric conditions. Considering the anatomy of the vascular

tree of the brain and what is known concerning the transmission of electrical currents through tissues, one might postulate that a high percentage of the current traversing the brain would concentrate on and be transmitted along the vascular tree of the brain or in its blood stream. According to this concept, only as the current concentrated on the vascular tree in the cortex or escaped into the deep subcortical portions of the brain from the deeper blood vessels would the brain be stimulated. Since the effect on the vascular tree might be reflected in changes of the semipermeable characteristics of the membranes composing the blood vessel walls, as indeed has been suggested by neuropathological studies, we have initiated studies using techniques previously reported for measuring alterations in the permeability of the blood-brain barrier. The permeability of the blood-brain barrier was therefore studied in the same manner as mentioned previously, both before and after a series of electroshock treatments in cats. It was found, as suspected, that electroshock therapy did appreciably increase the permeability of the blood-brain barrier and that this effect persisted for days in contradistinction to the fleeting change of permeability which occurs in nerve tissue after its electrical stimulation. In another series of experiments, in which the cats were injected with Trypan Red before the series of electroshock treatments were given, it was found that the dye prevented the permeability changes of the first series of experiments. In these same experiments, a generalized cerebral dysrhythmia was obtained by electroencephalography in the initial shock experiments but was either not obtained or only slightly so in the second series of experiments after Trypan Red was used.

Since Trypan Red appears to counteract both the increase in permeability of the blood-brain barrier and the cerebral dysrhythmia associated with electroshock therapy, one wonders if it might not also counteract the beneficial effect of such therapy in psychiatric conditions. Although the practical difficulties of clinical investigation along these lines are obvious, suitable methods are being sought to test further the possible relationship between these persistent neurophysiologic phenomena and the beneficial effects of electroshock therapy.

An increase in permeability presumably occurs with the abnormal vasodilatation such as is associated with headaches of vascular origin, such as migraine and histamine headaches. This factor may account for the pipe-stem arteries, prolongation of symptoms and ineffectiveness of ergotamine tartrate when given late in an attack of migraine, for example. Again, this same permeability mechanism is presumably involved in conditions associated with perivascular disease, such as encephalitis lethargica, anterior poliomyelitis, the demyelinating diseases and the more severe post-traumatic head conditions already mentioned. Since such perivascular disease probably plays at least a secondary role in the pathological physiology of these conditions, supravital dye therapy, if it diminished or controlled this effect, might prove of some therapeutic value in them. Certain possibilities along these lines are now being investigated and it is hoped will be the subject of more specific reports at a later date.

REFERENCES

1. Aird, R. B.: Mode of action of Brilliant Vital Red in epilepsy, Arch. Neurol. & Psychiat., 42:700-723 (Oct.), 1939.

2. Aird, R. B., and Strait, L. A.: Protective barriers of the central nervous system, an experimental study with Trypan Red, Arch. Neurol. & Psychiat., 51:54-66 (Jan.), 1944.

3. Aird, R. B., Cohen, W. E., and Weiss, S.: Convulsive action of triphenyl phosphite, Proc. Soc. Exper. Biol. & Med., 45:306-309, 1940.

4. Aird, R. B.: Preliminary report on Trypan Red therapy in amyotrophic lateral sclerosis, Tr. Am. Neurol. A., 170-172, 1946.

5. Aird, R. B., and Strait, L. A.: Neurophysiologic studies on experimental cerebral concussion, A. Research Nerv. & Ment. Dis., Proc. (1943), 24:283-295, 1945.

6. Aird, R. B., Strait, L. A., Hrenoff, M. K., and Zealear, D.: Neurophysiological studies on cerebral concussion, Tr. Am. Neurol. A., 1947.

7. Aird, R. B., Strait, L. A., Pace, J. W., Hrenoff, M. K., and Bowditch, S.: Neurophysiological effects of electroshock therapy, submitted for publication.

8. Cobb, S., Cohen, M. E., and Ney, J.: Brilliant Vital Red as an anticonvulsant, J. Nerv. & Ment. Dis., 85:438-441, 1937; Anticonvulsive action of Vital dyes, Arch. Neurol. & Psychiat., 40:1156-1177 (Dec.), 1938.

9. Evans, J. P., and Scheinker, I. M.: Histologic studies of the brain following head trauma: VI. Post-traumatic central nervous system changes interpreted in terms of circulatory disturbances, A. Research Nerv. & Ment. Dis., Proc. (1943), 24:254-273, 1945.

10. Friedmann, U.: Blood-brain barrier, Physiol. Rev., 22:125-145, 1942.

11. Katzenelbogen, S.: The cerebrospinal fluid and its relation to the blood: A physiological and clinical study, Baltimore, Johns Hopkins Press, 1935.

12. Scheinker, I. M., and Evans, J. P.: Histologic studies of the brain following head trauma: V. Alterations in the vessels of the central nervous system following trauma, A. Research Nerv. & Ment. Dis., Proc. (1943), 24:98-130, 1945.

13. Spatz, H.: Die bedeuting der vitalen färbung für die lehre vom stoffaustausch zwischen dem zentralnervensystem und dem übrigen körper, Arch. f. Psychiat., 101:267-358, 1934.